The Marginal Nuclei in the Spinal Cord of Reptiles: Intraspinal Mechanoreceptors[1]

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ABSTRACT. The marginal nucleus is a congregation of large cells in the lateral portion of the spinal cord and is found in a number of vertebrates including man and is prominent in reptiles. Recent studies suggest that these cells are mechanoreceptors that respond to stretching of the spinal cord, and are unique in that they are located within the central nervous system. Studies on the marginal nucleus in crotaline snakes show that the denticulate ligament forms an intimate part of this mechanoreceptive area. It undergoes structural changes within the intervertebral areas where stretching and bending of the vertebral column occur. This was also the case in two species of colubrid snakes that were studied, although some slight anatomical differences were noted within the marginal nucleus and the ligament. In lizards, the basic structure is also the same, but only the ventral portion of the ligament undergoes changes, and the marginal neurons are associated primarily with this portion of the ligament. In turtles, the ligament is considerably reduced in size, and the marginal nucleus is represented by only a few cells in the upper cervical regions of the spinal cord. Ultrastructural studies on the crotaline spinal cord show that the neurons extend dendrites into a lateral neuropil area, and that dendrites give rise to a number of small tubules, parallel to the spinal cord and ligament. Other ultrastructural features, characteristic of peripheral mechanoreceptors, are also found in this intraspinal mechanoreceptive area.

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INTRODUCTION

Receptors that respond to such stimuli as touch, pain, or temperature are usually associated with the peripheral nervous system. Only temperature-sensitive neurons had been described within the central nervous system (Simon 1974, Boulant 1981) until recently, when mechanoreceptors were also discovered in the spinal cord. Although the latter were described in lampreys (*Petromyzon* and *Ichthyomyzon,* Grillner et al. 1984), there is some evidence that they are found in a number of other vertebrates including man. These mechanoreceptors in lampreys co-ordinate rhythmic movements, but in other animals they may have different sensory functions. The spinal cord within the vertebral canal is stretched, compressed, and pulled in various directions as the body changes posture. Having receptors within the spinal cord itself, sensitive to these changes, could serve as an excellent means for feedback control or protection against excess movements. There is some evidence that marginal nuclei of the spinal cord appear to serve such a mechanoreceptor function. Furthermore, the denticulate ligament that helps to support the spinal cord within the canal appears to have a functional relationship to these receptors.

The presence of a group of neurons, usually called the marginal nucleus, in the lateral portion of the white matter of the spinal cord has been known for nearly 100 years, but it has been assumed that these neurons were related to the motor system. They are most prominent in reptiles and birds, but they are also found in some other vertebrates including mammals. Their sensory function has become evident through the research of Grillner et al. (1981), who have studied the innate rhythmic coordinated movements in a variety of vertebrates. While studying lamprey spinal cord, they became aware that this coordinated movement could be elicited by mechanically bending the spinal cord even if all of the dorsal and ventral roots were sectioned. This suggested that there are mechanoreceptors within the spinal cord (Grillner et al. 1982); subsequent anatomical and electrophysiological studies supported this observation (Grillner et al. 1984).

Marginal nuclei have been described in reptiles: in lizards by von Kölliker (1902) and Terni (1926); in snakes (*Trigonocephalus* and *Tropidonotus*) by Shimada (1912); in the alligator, Gaskell (1885); and in the turtles by Ariens Kappers et al. (1936). In birds, the marginal nuclei are more complex. One of the earliest descriptions was by Lachi (1889) who noticed these nuclei especially in the lumbar region where they form a protruding lobe, often now referred to as accessory lobe of Lachi. These marginal nuclei were also described in birds by von Kölliker (1902), Streeter (1904), and Huber (1936). In mammals, marginal nuclei were first described by Polyak (1924) in bats (*Myotis, Rhinolophus, Pipistrellus*), where several large cells are located at the edge of the spinal cord. Duncan (1953) described cells in the lateral funiculus of the cynomolgus monkey; but these subpial cells were present primarily in the lumbar segment. Of interest also are the studies of cats and humans where marginal cells are found throughout most segments of the spinal cord near the attachment of the denticulate ligament (Anderson et al. 1964)

Shimada (1912) noted that the marginal cells in snakes were always located near the denticulate ligaments. I had also wondered if there was a functional relationship between these cells and the ligaments and after reading Grillner et al. (1981, 1982, 1984), I concluded that this was not a coincidence, but that the ligament in some way contributed to the receptor mechanism. Detailed studies of crotaline snakes were undertaken, some of which have been published elsewhere (Schroeder 1984, Schroeder and Richardson 1985). To establish whether similarities were present among other reptilian species, two additional species of snakes, a lizard, and turtle were also examined.

MATERIALS AND METHODS

The experimental animals were five *Crotalus viridis* (eastern diamondback rattlesnake); two *Nerodia sipedon* (northern water snake); two *Coluber constrictor* (black racer); one *Tubinambis nigropunctatus* (Tegu lizard); and one *Pseudemys scripta* (red-eared turtle). The snakes

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and turtles were anesthetized with metofane (methoxyflurane, Pittman-Moore) and the lizard with an intraperitoneal injection of sodium pentobarbitol (24 mg/kg body weight). The rattlesnakes, water snakes, and turtle were fixed initially with a vascular rinse of saline, followed by a fixative consisting of 0.5% paraformaldehyde and 2.5% glutaraldehyde in phosphate buffer (pH 7.4). The black racers and lizard were fixed with 10% formaldehyde. After the spinal cord was removed, small tissue pieces were postfixed in 2% osmium tetroxide and embedded in epon. One-micron-thick sections were stained with toluidine blue. Thin sections, stained with uranyl acetate followed by lead citrate, were examined with a Hitachi H-300 electron microscope.

RESULTS

In snakes, the denticulate ligaments, one on each side of the spinal cord, are attached to the skull and extend to the tip of the tail where they adhere closely to the spinal cord at its ventrolateral edge. The ligaments generally contain collagen, fibroblasts, and elastic fibers; however, at the intervertebral areas the character of the ligament changes. The collagen is discontinued, and only fibroblasts and elastic fibers remain (Fig. 1). Precisely adjacent to this area, large neurons within the spinal cord are aligned along the width and length of this "weakened" portion of the ligament. These large neurons in a row are the most striking feature of this area. Between these neurons and the ligament is the lateral neuropil area; the medial neuropil area is on the mediodorsal side of the large neurons. In *Crotalus* this main receptor area is about 100 μm long and about 350 μm wide in a dorsoventral direction and extends about 1 mm in a rostrocaudal direction.

The spinal cords of *Nerodia sipedon* and *Coluber constrictor* were also examined. In general, *Nerodia* receptor areas were similar to that of *Crotalus* (Fig. 2). Even under the dissecting microscope, the segment of the ligament that loses the collagen was obvious as a band across the ligament. On gross inspection the collagen-free area in the ligament of *Coluber* was not evenly spaced across the ligament as in the other two species but appeared to zigzag. This became clearer upon examination of the cross sections. In the rostral portion of the receptor area, only the ventral portion of the ligament loses the collagen, whereas in the more caudal receptor area, only the dorsal portion of the ligament is collagen free. The spinal cord of the Tegu lizard was also examined. The denticulate ligament of the spinal cord appears to be quite similar to that of snakes. The intervertebral segment of the ligament where the collagen disappears can also be detected with a dissecting microscope. In contrast to the snakes, this segment is not a band across the ligament, but appears to be located only half-way across. When the cord was sectioned, the following became apparent (Fig. 3). In cross-section, the ligament never loses all of its collagen but takes on a comma-shaped appearance, with the dorsal portion remaining filled with collagen and only the ventral portion becoming thinner and containing only the elastin. It is only in this ventral portion that the large neurons and most of the neuropil are located. Only the cervical segments of the turtle were examined. The denticulate ligament is relatively smaller, and there does not appear to be an area that loses its collagen. A rostral cervical area shows a thin strand of neuropil near the ligament, but only one large neuron was seen (Fig. 4). Caudal cervical areas were serially sectioned and examined for marginal nuclei, but none were seen. The presence of these marginal nuclei only in the upper cervical areas could be explained by the restricted movements of the neck of the turtle. This organism has a wide range of head and upper neck movements; however, neck movement near the carapace is quite limited.

An ultrastructural study of the crotaline receptor area was also done and a brief description follows. The large neurons have Nissl substance scattered throughout the cytoplasm, numerous mitochondria, and other standard cytoplasmic inclusions such as microtubules and Golgi apparatus (Fig. 5). The neurons located along the width of the ligament extend dendrites into the lateral neuropil located between their cell bodies and the ligament. These dendrites have multivesicular bodies, a greater concentration of microtubules and many mitochondria. The latter are often so numerous that there is very little space for cytoplasm. Along the proximal sides of the dendrites and the soma are synapses. These synaptic boutons, as well as most of the other boutons in the neuropil, contain clear round vesicles. At the blunt tip of the dendrites, however, small tubules emerge in a rostral or caudal direction, parallel to the length of the spinal cord and the denticulate ligament (Fig. 6). These processes are always in close proximity to an axon-like structure. The small tubules all have an approximate diameter of 0.12 μm. Their length is difficult to determine since they usually leave the plane of section. However, they could be followed for at least 5 μm. The only structure within these small tubules appears to be neurofilaments. The axon-like structures have variable diameters ranging from about 0.75 μm to 1.0 μm and a variety of inclusions. Microtubules and neurofilaments are distributed evenly throughout their cytoplasm. There are also patches of small, clear vesicles usually at the edge of the process, and occasionally elongated mitochondria. The origin of axon-like structures has not been determined.

Towards the medial side of the receptor area, smaller neurons extend processes into the medial neuropil. Many boutons, primarily with clear vesicles, synaptic areas, and unmyelinated axons are found here. The small tubules and axon-like structures found in the lateral neuropil are absent in this region.

DISCUSSION

The anatomical data on the marginal cells and denticulate ligament of the snake suggest that this is a mechanoreceptive area. The ligament changes in character precisely at the area where movement occurs between the vertebrae. It is no longer supported by collagen, but only by elastin (and ground substance) which causes a "weakening" of the ligament which can then stretch and bend quite easily. This would help to narrow the stimulus to a precise area within the cord and probably enhance the stimulus as well. Any stretching (or bending) would affect the small tubules within the lateral neuropil which run parallel with the ligament. By some yet unknown mechanism this information could be passed on to the dendrites of the receptor cells. That the marginal cells are mechanoreceptors is also based on the fact that the neuronal processes of snakes are very similar in structure to those of neuronal processes found in most other peripherally-located mechanoreceptors.

The neuronal elements of all peripheral mechanoreceptors have several features in common. They are rich in mitochondria, microtubules, multivesicular bodies, and other cytoplasmic inclusions. They also have

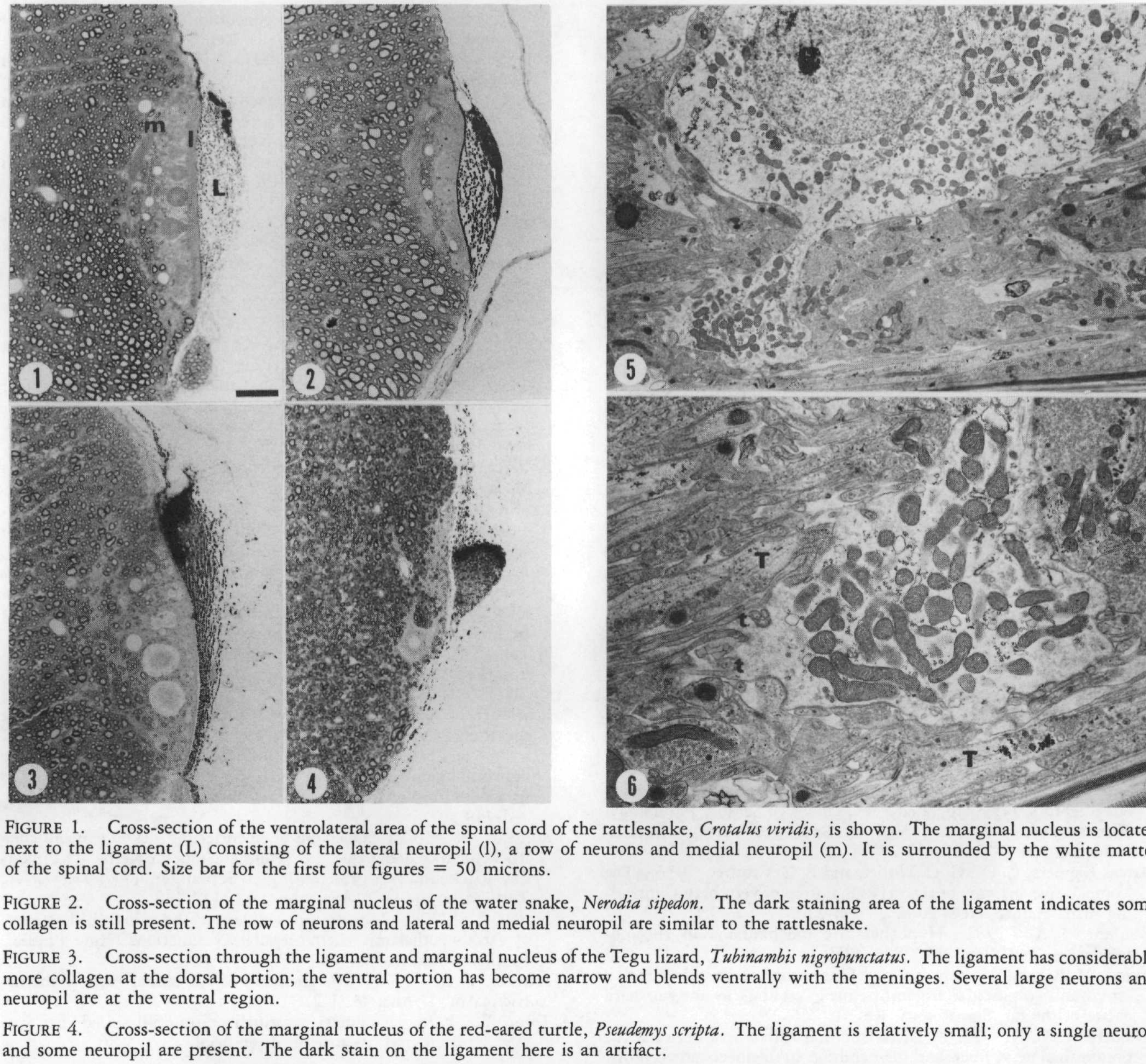

FIGURE 1. Cross-section of the ventrolateral area of the spinal cord of the rattlesnake, *Crotalus viridis*, is shown. The marginal nucleus is located next to the ligament (L) consisting of the lateral neuropil (l), a row of neurons and medial neuropil (m). It is surrounded by the white matter of the spinal cord. Size bar for the first four figures = 50 microns.

FIGURE 2. Cross-section of the marginal nucleus of the water snake, *Nerodia sipedon*. The dark staining area of the ligament indicates some collagen is still present. The row of neurons and lateral and medial neuropil are similar to the rattlesnake.

FIGURE 3. Cross-section through the ligament and marginal nucleus of the Tegu lizard, *Tubinambis nigropunctatus*. The ligament has considerably more collagen at the dorsal portion; the ventral portion has become narrow and blends ventrally with the meninges. Several large neurons and neuropil are at the ventral region.

FIGURE 4. Cross-section of the marginal nucleus of the red-eared turtle, *Pseudemys scripta*. The ligament is relatively small; only a single neuron and some neuropil are present. The dark stain on the ligament here is an artifact.

FIGURE 5 and 6. Electron micrograph of a longitudinal section of the marginal nucleus of the rattlesnake, *Crotalus viridis*.

FIGURE 5. Neuron, with nucleus and nucleolus, extends a dendrite into the lateral neuropil which is enlarged in the next figure. The ligament is at the bottom on the right. ×6,000.

FIGURE 6. Dendritic process containing many mitochondria. Synapses are along the upper portion of the dendrite; small tubules emerge from the lower left (t). Axonlike structures (T) are also in this section. The ligament is at the bottom on the right. ×15,000.

finger-like extensions into the surrounding environment (Munger 1971, Andres and von During 1973, Chouchkov 1976, 1978, Iggo and Andres 1982, Byers and Yeh 1984). The dendritic processes of the large cells in the spinal cord of the snake match this description very well. Furthermore, receptors such as Pacinian and Herbst corpuscles have numerous cytoplasmic lamellae of modified Schwann cells surrounding the neuronal terminal, often with desmosome-like contacts. The transducer sites are thought to be the finger-like processes that project from the sensory ending between the lamellae. Mechanical deformation of the lamellar system could then be transmitted to the mechanoreceptor nerve ending. The small tubules that emerge from the dendrites of the large neurons of snakes are very similar to the finger-like processes of peripheral mechanoreceptors; however, instead of being associated with lamellae of Schwann cells, they are near the "weakened" ligament that focuses the stimulus to this area. Byers and Yeh (1984) speculated that the thin filaments found within the finger-like processes of neuronal endings could be actin and analogous to the stereocilia of hair cells that are known to be involved in sensory transduction mechanisms. Therefore, movement of the finger-like processes might generate action potentials.

The exact function of the intraspinal mechanoreceptors is still speculative at this time. The only electrophysiological data available are those of Grillner et al.

(1984) who recorded from the edge cells of the isolated spinal cord of the lamprey. When the lateral edge was stretched slowly, the cell depolarized and began to spike. When the lateral edge was released from stretch, the membrane potential returned to the previous level. These edge cells have dendrites that ramify along the lateral border of the spinal cord. Grillner et al. (1984) suggested that when stretch is applied along the lateral margin of the spinal cord, as during swimming, the processes extending along the lateral margin are deformed, causing a stretch-evoked depolarization of edge cells.

The differences found in the marginal cells of several species of reptiles suggest that a careful study must be made of each species before conclusions can be made. Even among the three species of snakes there were some structural differences apparent at the light microscope level. Because the Tegu lizard represents only one species of lizard, conclusions cannot be made therefore about the anatomy of marginal nuclei in other lizards. However, the similarities to the marginal nuclei of the snake suggest that intraspinal mechanoreceptors may play an important role in their behavior as well. The limited movement of the vertebral column of turtles is reflected in the reduced size of the marginal nuclei. Their presence at the upper cervical levels, where the greatest movement occurs, adds credence to the conclusion that marginal nuclei are mechanoreceptors.

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John Carroll faculty play an active role in the Great Lakes Symposium, August 6, 1986. Speakers and their topics include:
John Piety — Discovery and exploration of the Great Lakes
Andrew M. White — History and status of the Lake Erie fishery
Kathleen L. Barber — Who's responsible for the Great Lakes?
Edward Skoch — Life on the North Coast